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Original Article

Microbiology Section

Emergence of Drug Resistant *Enterococcus* Species from Patients with Urinary Tract Infections in a Tertiary Care Centre- A Retrospective Analysis

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ABSTRACT

Introduction: Urinary Tract Infection (UTI) is one of the most common infections, among patients who are hospitalised. Enterococci are one of the frequent isolates among UTI patients, gaining considerable clinical importance, due to their escalating drug resistance, affecting debilitated patients or patients with prolonged hospital stay. Among Multi-Drug Resistant (MDR) enterococci, glycopeptide-resistant enterococci are being increasingly reported. The intrinsic resistance of enterococci and their ability to acquire and disseminate antibiotic resistant genes to other organisms pose a challenge in the treatment of enterococcal infections with MDR.

Aim: To investigate the prevalence of MDR, Extensively-Drug Resistant (XDR) and Pan-Drug Resistant (PDR) Enterococci spp. isolated from urine samples in a tertiary care centre.

Materials and Methods: The present retrospective study was conducted to find the burden of drug resistant *Enterococcus* spp. in urine samples, analyse their antibiogram and patients' socio-demographic information from January to December 2019. Records of microscopic observations to antibiogram of each isolate was noted down from the register for urine samples, and further analysed. The data was coded, verified, entered and analysed using Statistical Package for Social Sciences (SPSS) version 18.0.

Results: The majority of patients 127 (20%) belonged to the age group of 21-30 years of age. Among 10,535 urine samples received, 635 culture isolates were identified as *Enterococcus* spp., of which 17.95%, 3.30% and 0.62% were identified as MDR, XDR and PDR enterococci, respectively. The antimicrobial susceptibility was found to be least for High Level Gentamicin (HLG) (66.14%), and penicillin (68.81%), followed by ampicillin (81.10%) and nitrofurantoin (86.40%). Twenty eight isolates (7.9%) were identified as Vancomycin Resistant Enterococci (VRE), 9 (2.5%) as linezolid resistant and 12 (3.4%) as linezolid intermediate enterococci.

Conclusion: Identification and prevention of the alarming increase in MDR, XDR and PDR enterococci spp. is cardinal to prevent morbidity and mortality of affected patients. Analysis of antibiogram periodically, is important in selection of appropriate drugs to prevent the incoherent use of antibiotics.

Keywords: Antibiogram, Extensively-drug resistant, Multi-drug resistance, Pan-drug resistance, Vancomycin resistant enterococci

INTRODUCTION

Enterococci are a part of the normal intestinal flora, colonising the oral cavity, genitourinary tract, and the perianal area [1-3]. However, over the last two decades they have emerged as the most common cause of nosocomial infections, keeping in pace with the development of antimicrobial resistance against the newest range of drugs currently available such as with the lipopeptide daptomycin, or a combination of daptomycin with ampicillin, a combination of ampicillin with ceftriaxone [1,4-7]. Enterococci are frequently recovered from UTI, which are among the most commonly encountered infections among hospitalised patients, following severe illness with prolonged hospitalisation and multiple antibiotic treatment [8-10]. The presence of intrinsic resistance mechanisms in enterococci which makes them antagonistic to routinely used antibiotics such as cephalosporins, aminoglycosides, trimethoprim-sulfamethoxazole, beta-lactams, lincosamides, and their disposition to acquire resistance have made them an enormous challenge for clinicians in the recent decade [11-14].

The resistance to currently available antibiotics, leads to limited treatment options and results in natural selection and spread of MDR enterococci in the hospital environment. Further the increasing resistance to vancomycin calls for the need of new treatment modalities with last resort antibiotics such as linezolid and daptomycin as there have been many reports of *Enterococcus faecalis (E. Faecalis)* developing resistance against linezolid [13,15-20]. "MDR is defined as non susceptibility to at least one agent in three or more antimicrobial categories. Non susceptibility to at least one agent in all but two or fewer antimicrobial categories is defined as XDR. PDR is defined as non susceptibility to all agents in all antimicrobial categories [17]."

Prolonged stay in hospital, increased use of antibiotics, particularly in immunosuppressed or immunocompromised patients have put them at the risk of developing MDR enterococcal infections resulting in high mortality rates [21-25]. Antimicrobial susceptibility testing and analysis of the antibiogram may facilitate the formulation of new therapeutic approach against enterococcal infections, and aid in eliminating the extensive usage of last resort drugs as empirical treatment. Therefore, this study aim to retrospectively detect the burden of VRE, MDR, XDR and PDR enterococci isolated from urinary samples from patients in a tertiary care centre during a time period of one year.

MATERIALS AND METHODS

A retrospective analysis of prevalence and antimicrobial resistance of enterococci isolated from urine samples of patients received in microbiology laboratory, in a tertiary care centre, were performed. All

enterococci isolates were obtained from urine samples for a period of one year, from January 2019 to December 2019 and analysed and interpreted during the month of January 2020. Retrospective data was taken from the secondary data available as records maintained for isolation of organisms and their antimicrobial susceptibility pattern in urine samples. The Institutional Ethics Committee (IEC) approval was obtained from the Ethical committee with the ethical clearance number 2021/IEC/2020.

Inclusion criteria: All patients' urine samples tested in the Central laboratory were included in the study. Information regarding the patients' age, gender, ward type, and clinical conditions were included in the study as well.

Exclusion criteia: Samples other than urine and isolates other than enterococci were excluded from the study.

Microbiological Examination

Mid-stream urine samples collected under aseptic conditions, were received by the laboratory which were processed by microscopic examination by wet mount method and cultured on Chromogenic (CHROM) Agar and 5% sheep blood agar to isolate the aetiological agents. The identification of the isolates was further confirmed by biochemical tests and antimicrobial susceptibility tests. Gram-negative organisms were identified by mannitol motility medium, triple sugar ion, indole, Simmons' citrate and Christenson's urease tests and gram-positive organisms were identified by catalase, coagulase, and bile esculin agar tests [1,25]. Differentiation of *Candida albicans* from non-*albicans Candida* was performed by germ tube test, colour differentiation produced in *Candida* Chrome Agar (CCA) and by their arrangement and morphology on Corn Meal Agar (CMA).

Antimicrobial Susceptibility Tests

Antimicrobial susceptibility test was performed following Kirby Bauer's disc diffusion test according to Clinical and Laboratory Standards Institute (CLSI) guidelines [26]. The commercially available antibiotic discs employed for susceptibility test include the following: ampicillin (10 µg), gentamicin (10 µg), nitrofurantoin (300 µg), penicillin (10 units), ciprofloxacin (5 µg), HLG (120 µg), tigecycline (15 µg), imipenem (10 µg), vancomycin (30 µg), teicoplanin (30 µg), and linezolid (30 µg). A zone of ≥12 mm with sharp zone edges were considered to be sensitive and zone diameter of <12 mm with fuzzy zone edges to be resistant for vancomycin disks. For linezolid disks, a zone diameter of ≥19 mm was taken to be sensitive, 17 to be intermediate and <16 to be resistant [26].

The antimicrobial susceptibility test was performed by disk diffusion test as per the standard guidelines mentioned in the CLSI [26]. The cultured plates were incubated for 16-18 hours at 35°C and the results were interpreted by measuring the inhibition zone around each antibiotic disc. They were further classified as MDR non susceptible to ≥1 agent in ≥3 antimicrobial categories, XDR non susceptible to ≥1 agent in all but ≥2 categories and PDR non susceptible to all antimicrobial agents [27].

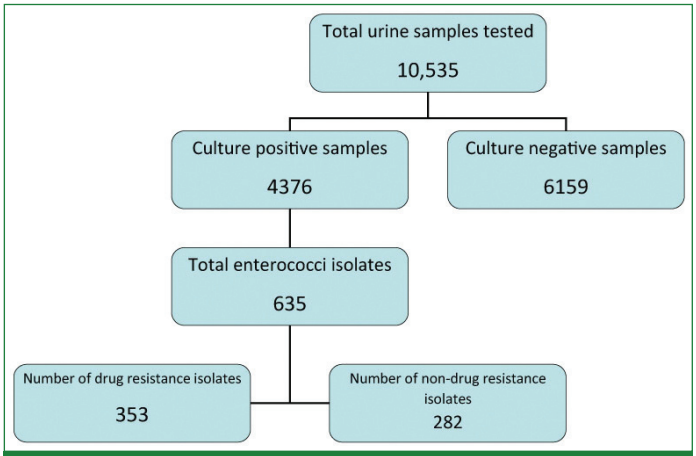
Epsilometer test (E-test) was performed for vancomycin and linezolid on all isolates that showed resistance to vancomycin and linezolid in disk-diffusion test as per standard CLSI guidelines [26]. Similar to disk-diffusion test, swabs with enterococci inoculum were lawn-cultured on the surface of sterile Mueller Hinton Agar (MHA) medium and the E-strip was placed in the centre. The cultured plates were incubated for 18-24 hours at 37°C and the results were interpreted by the reading the Minimum Inhibitory Concentration (MIC) at the intersection of the lower part of the ellipse-shaped growth inhibition area.

STATISTICAL ANALYSIS

Statistical analysis was done on Microsoft Excel. The data was coded, verified, entered and analysed using SPSS version 18.0.

RESULTS

During the study period a total of 10,535 urine samples were analysed, among which 4376 samples were found to be culture positive [Table/Fig-1]. Within the culture positive samples, 635 isolates were identified as *Enterococcus* species. Of the total isolates, 388 (61%) was procured from hospitalised patients, 216 (34%) from in-patients, and 96 (15.1%) from outpatients. Majority of the isolates were identified in the age group of 21-30 years followed by 51-60 years [Table/Fig-2]. Wet mount microscopic observation revealed that 2374 (22.5%) samples had few pus cells with bacteria, 1760 (16.7%) had occasional pus cells with bacteria and 577 (5.5%) sample had moderate pus cells with bacteria. Majority of samples (42.7%) showed occasional pus cells without any organisms.

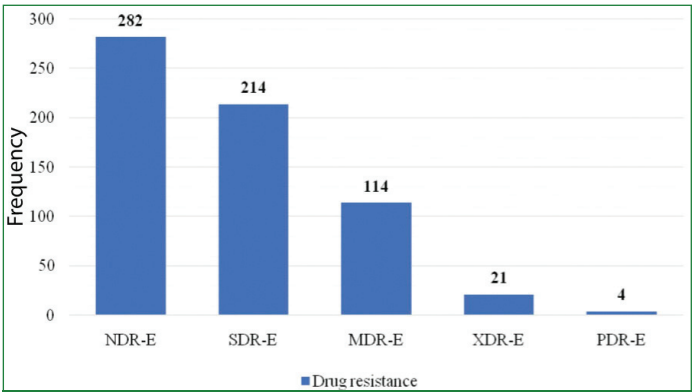


[Table/Fig-1]: Total sample distribution.

Age group (years)	VRE (%)	LRE (%)	MDR (%)	XDR (%)	Total no. of isolates (%)
0-10	0 (0)	1 (11.1)	6 (5.26)	0 (0)	27 (4.25)
11-20	2 (7.1)	1 (11.1)	4 (3.50)	1 (4.76)	44 (6.92)
21-30	2 (7.1)	0 (0)	6 (5.26)	1 (4.76)	127 (20)
31-40	0 (0)	0 (0)	12 (10.52)	0 (0)	79 (12.4)
41-50	5 (17.8)	0 (0)	15 (13.15)	3 (14.23)	76 (11.96)
51-60	7 (25)	4 (44.4)	26 (22.8)	7 (33.3)	107 (16.85)
61-70	8 (28.5)	1 (11.1)	28 (24.5)	4 (19)	105 (16.53)
71-80	3 (10.7)	2 (22.2)	16 (14)	4 (19)	57 (8.97)
81-90	1 (3.5)	0 (0)	1 (0.8)	1 (4.76)	13 (2)
Total	28 (4.4)	9 (1.4)	114 (17.9)	21 (3.3)	635

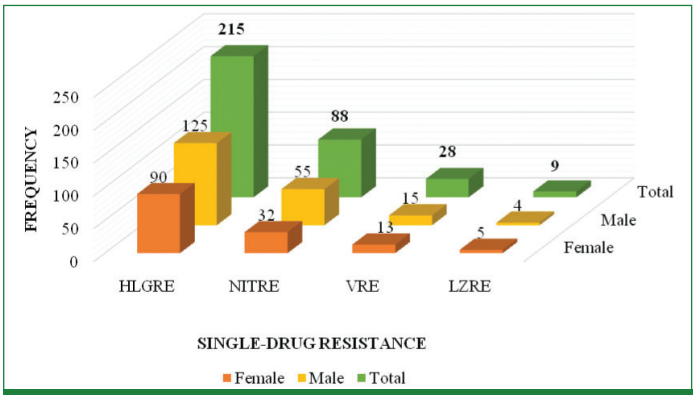
[Table/Fig-2]: Distribution of isolates among various age groups.
VRE: Vancomycin resistant enterococci; LRE: Linezolid resistant enterococci; MDR: Multi-drug resistant isolates; XDR: Extensive drug resistant isolates

Among the total 635 isolates identified, 282 (44.4%) did not exhibit resistance to any of the antibiotics tested and were categorised as non drug resistant isolates [Table/Fig-3]. Among single drug

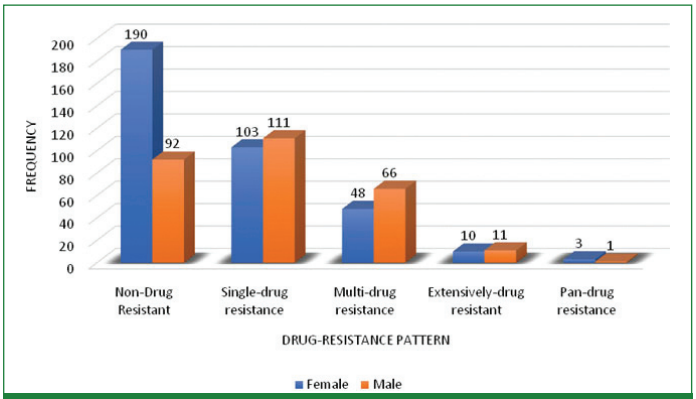


[Table/Fig-3]: Prevalence percentage of AST pattern of enterococci isolated from urine samples.
NDR-E: Non-drug resistant enterococci; SDR-E: Single drug resistant enterococci; MDR-E: Multidrug resistant enterococci; XDR-E: Extensive drug resistant enterococci; PDR-E: Pan drug resistant enterococci

resistant isolates, the highest drug resistance was observed with HLG [Table/Fig-4]. Non-drug resistant isolates were higher in females 190 (67.4%) compared to males 92 (32.6%) [Table/Fig-5], whereas almost all types of drug resistant isolates (except PDR isolates, which were higher in females) were predominantly identified in males.

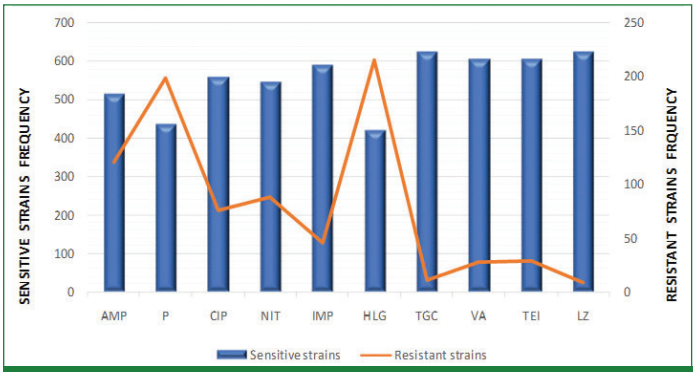


[Table/Fig-4]: Distribution of single drug resistance for significant antibiotics by gender. LZRE: Linezolid resistant enterococci; VRE: Vancomycin resistant enterococci; NITRE: Nitrofurantoin resistant enterococci; HLGRE: High level gentamicin resistant enterococci



[Table/Fig-5]: Distribution of drug-resistance pattern by gender.

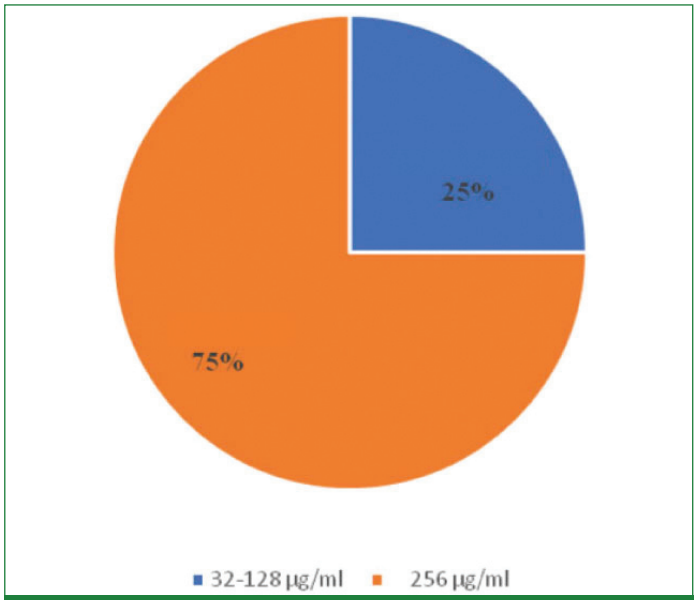
The highest resistance was observed for HLG (66.14%) and penicillin (68.81%), followed by ampicillin (81.10%), nitrofurantoin (86.40%) and ciprofloxacin (88.03%) [Table/Fig-6]. Intermediate drug sensitivity was also observed for linezolid 12 (3.4%), vancomycin 5 (1.4%), and ampicillin 3 (0.8%) among the enterococcal isolates, more prevalent in hospitalised patients and patients in emergency care units. The drug resistant isolates were more commonly isolated among hospitalised patients followed by patients in emergency care compared to the outpatients [Table/Fig-7]. Most of the isolates 18 (64.2%) had a MIC of ≥256 µg/mL [Table/Fig-8]. Among the study patients, Type 2 Diabetes Mellitus was the most common clinical diagnosis 89 (14%), followed by Chronic Kidney Disease (CKD) 57 (9%). Enterococci with co-infections 125 (19.6%) were also noted, among which the common co-infection was caused by *Escherichia coli* followed by *Escherichia coli* (Extended Spectrum Beta Lactamase), *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* [Table/Fig-9].



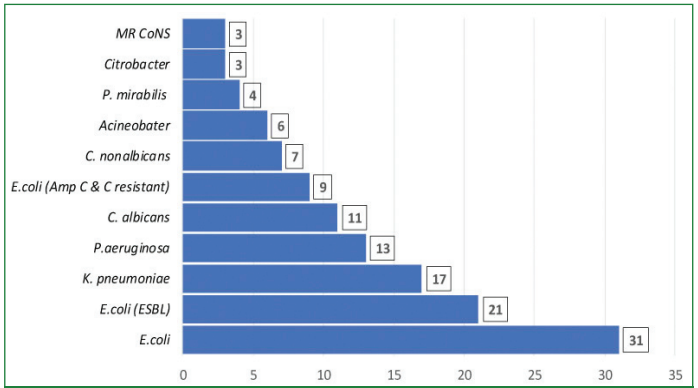
[Table/Fig-6]: Frequency of sensitive and resistant strains for each antibiotic drug. AMP: Ampicillin; P: Penicillin; CIP: Ciprofloxacin; NIT: Nitrofurantoin; IMP: Imipenem; HLG: High level gentamicin; TGC: Tigecycline; VA: Vancomycin; TEI: Teicoplanin; LZ: Linezolid

Out-patients (OP)	*Enterococcus* spp. isolated (%)	MDR-E isolates (%)	XDR-E isolates (%)
Total	96 (15.1%)	5 (4.4%)	1 (4.8%)
In-patient wards (IP)	***Enterococcus* spp. isolated (%)**	**MDR-E isolates (%)**	**XDR-E isolates (%)**
Urology	25 (15.6%)	7 (11.5%)	0 (0)
Nephrology	11 (6.8%)	8 (13.1%)	3 (23%)
General Medicine	67 (41.8%)	30 (49.2%)	7 (53.8%)
General surgery	25 (15.7%)	10 (16.4%)	1 (7.7%)
Casualty	13 (8.2%)	5 (8.2%)	1 (7.7%)
Obstetrics and Gynaecology	14 (8.7%)	1 (1.6%)	1 (7.7%)
Labour	5 (3.2%)	0 (0)	0 (0)
Total	160	61	13
Emergency wards (E)	***Enterococcus* spp. isolated (%)**	**MDR-E isolates (%)**	**XDR-E isolates (%)**
IMCU	22 (39.3%)	17 (43.6%)	3 (50%)
Respiratory ICU	16 (28.6%)	9 (23%)	2 (33.3%)
Surgical ICU	8 (14.3%)	5 (12.9%)	1 (16.7%)
Paediatric ICU	1 (1.8%)	1 (2.6%)	0 (0)
PACU	3 (5.4%)	2 (5.1%)	0 (0)
Cardiac ICU	1 (1.8%)	1 (2.6%)	0 (0)
Cardio-thoracic ICU	3 (5.2%)	2 (5.1%)	0 (0)
Step down ICU	2 (3.6%)	2 (5.1%)	0 (0)
Total	56	39	6

[Table/Fig-7]: Distribution of drug-resistant isolates by wards. IMCU: Intensive medical care unit; ICU: Intensive care unit; PACU: Postanesthesia care unit



[Table/Fig-8]: Distribution of Minimum Inhibitory Concentrations (MIC) among the vancomycin resistant isolates (28 isolates).



[Table/Fig-9]: Frequency of co-infections in patients associated with Enterococci spp. X-axis denotes the co-infection causing organisms.

DISCUSSION

Enterococci, historically considered as a second-rate pathogen, has emerged as the second leading nosocomial pathogen and the third common cause of bacteremia [4,7]. The increasing exploitation of antibiotics have led to emergence and spread of antimicrobial resistance, challenging the current therapeutic modalities [1,8-10]. This study focuses on the emerging drug resistance in *Enterococcus* spp. isolated from urine samples and their distribution among patients.

In present study, majority of the isolates were obtained from the age group 21-30 years followed by 51-60 years. However, in other studies [1,4,6-9] high percentage of isolates were obtained from neonates and age group <20 years, followed by 21-30 years. Of the total 635 isolates, the study showed a female preponderance consistent with some studies [6,12], while Saraswathy MP, reported a male predominance of 61% among 100 isolates [1].

Three-hundred eighty-eight (61.1%) hospitalised patients, {particularly in general medicine 67 (41.87%) followed by surgery and urology wards 25 (15.6%)} and 56 (8.81%) patients in emergency care were more susceptible to UTI caused by both non-drug resistant and drug resistant isolates of enterococci as compared to 96 (15.1%) out-patients. This data is consistent with many studies [1,2,4,12-13]. Enterococci was isolated with a mixture of two or three organisms (19.6%), among which *E. coli*, *E.coli* (ESBL) were profound, followed by *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, which is similar to other reports [1], although the enterococci isolated in pure forms in present study was comparatively higher 510 (80.3%).

The antibiogram of enterococci isolated in present study correlated with the reports of Karna A et al., [4]. Saraswathy MP reported similar antibiogram in their study, wherein *E. faecalis* isolates were sensitive to vancomycin (100%), ofloxacin (61%), ciprofloxacin (59%), erythromycin (46%), amikacin (41%), tetracycline (36%) and HLG (77%) [1]. Many other studies have reported high percentage of resistance for ciprofloxacin, penicillin, ampicillin, and HLG [2,7,8,12,13]. HLG resistant isolates (33.86%) in present study were comparable with other studies [2,13,14]. Bhatt P et al., observed 32% HLAR by Kirby Bauer disk diffusion method compared to 39% HLAR by E-test method [2].

Vancomycin resistance was observed in 28 (6%) isolates by Kirby Bauer DDT (disk diffusion test) and their respective MICs were determined by E-test method. Most of the isolates showed a high MIC of ≥256 µg/mL, confirming their high resistance to vancomycin. Similar observations were reported in other studies [2,14]. A study conducted by Manimala E et al., reported 48% VRE isolation by Disk Diffusion Test (DDT) while only 4% VRE isolation by E-test method [13]. Of the four isolates two were susceptible to HLG, while in present study, only 5 (17.8%) VRE were susceptible to HLG. Although, author report a higher incidence of HLGR compared to VRE, a study conducted by Karna A et al., report otherwise, with 20.9% VRE compared to 18.7% HLGR isolates [4].

Linezolid, a broad-spectrum antimicrobial agent, is invaluable in infections caused by VR gram positive organisms [22]. In this study, 9 (1.4%) LRE and 12 (1.8%) intermediately resistant isolates were identified. Bhatt P et al., observed linezolid resistance in 2% of their isolates [2]. Over a period of eight years (2007-2014) Bagga B et al., had observed a consistent decrease in linezolid susceptibility in their hospital [22]. They reported a high incidence of non-susceptibility (54%) to linezolid as of 2014.

In the study we identified the following: 33.70% were SDR, 17.95% MDR, 3.30% XDR and 0.62% PDR isolates. Bhatt P et al., had reported a high prevalence of MDR isolates (63%) in their study [2], similar to other studies which reported high incidence of MDR isolates [3,4,28]. In a study by Asma R et al., 1% SDR, 4.1% MDR and 0.3% XDR isolates were reported with a high prevalence of drug resistant isolates in males compared to females [6].

This study signifies the profound influence of emerging drug resistant isolates of enterococci on hospitalised and emergency care patients. It is mandatory to define the species and antimicrobial susceptibility pattern of clinical isolates to determine the therapy and prevent further spread of the resistant strains in the hospital environment. Enterococci have emerged as the major cause of nosocomial UTI, and bacteremia, which necessitates the implementation of antibiotic stewardship and infection prevention and control policies stringently.

Limitation(s)

Since, the study was a retrospective one, the MICs of the isolates checked with E-strips could not be confirmed by microbroth dilution method. E-strip tests contained only values up to 256 µg/mL, thus resistant isolates with a higher MIC could not be determined. Speciation of the isolates was not performed. Further investigation of the genes responsible for vancomycin resistance could not be determined.

CONCLUSION(S)

This study reports the burden of emerging drug resistance to various antimicrobials in enterococci species isolated from urine samples of patients attending a tertiary care centre. Increase in MDR isolates and emergence of XDR and PDR were seen in the tertiary care centre. Hospitalised patients were found to be more susceptible to drug resistant infections, indicating the need to follow stringent infection control programmes in the hospital to prevent further emergence of resistance and spread of drug resistant isolates. The emergence of drug resistant isolates along with co-infections poses challenges to treatment and necessitates the significance of new therapeutic modalities and judicial use of antimicrobials.

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